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MOLECULAR TOXICOLOGY

Acetylcholinesterase inhibition: does it explain the toxicity of organophosphorus compounds?

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Abstract The hypothesis that acetylcholinesterase (AChE) inhibition is the mechanism of toxicity of organophosphorus (OP) compounds was examined by mathematically modeling the in vivo lethal effects of OP compounds and determining the amount of variation in OP toxicity that is explained by AChE inhibition. Mortality dose–response curves for several OP compounds (i.e., VX, soman, cyclosarin, sarin, tabun, diisopropylfluorophosphate and paraoxon) exhibited steep probit slopes (> 9.6) in guinea pigs. Steep probit slopes were also observed when the mortality dose–response curves for soman were examined in mice, rats, rabbits and non-human primates. The consistently steep probit slopes of the dose–response curves for highly toxic OP compounds suggested that these compounds have a single specific mechanism of toxicity regardless of the OP compound or the species in which it was tested. Regression analysis indicated that 93% of the 3,280-fold variation in the median lethal doses (i.e., LD_{50}) of OP compounds in rats was explained by the variation in their in vitro rate constants for inhibition of AChE. Conversely, 91% of the 23-fold variation in the ability of the oximes pralidoxime and obidoxime to protect against the toxicity of OP compounds in guinea pigs was explained by the variation in the in vitro ability of oximes to reactivate OP-inhibited AChE. The best explanation for this variety of observations was that the primary mechanism of in vivo toxicity for highly toxic OP compounds is the inhibition of AChE, and the residual unexplained variation in OP toxicity that might be explained by other mechanisms represents < 10% of the total variation in OP toxicity.

Keywords Organophosphorus · Acetylcholinesterase inhibition · Oxime reactivation · Dose–response · Toxic mechanism

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Introduction

Although the in vitro effect of organophosphorus (OP) compounds on acetylcholinesterase (AChE) has been extensively studied (De Jong and Benschop 1988), the hypothesis that OP inhibition of AChE is the primary mechanism of acute in vivo OP toxicity has recently undergone re-examination. The acute toxicity of OP compounds has traditionally been attributed to inhibition of AChE because the pathophysiological effects of these compounds (e.g., bradycardia, increased tracheobronchial secretions, bronchoconstriction, seizures and respiratory depression) are consistent with a cholinergic mechanism of toxicity (Karczmar 1970; Marrs 1993; Taylor 2001). However, several recent papers have suggested that OP compounds have direct toxic effects on other enzymes, acetylcholine receptors and receptor/channel complexes that are independent of AChE inhibition (Sultatos 1994; Pope 1999; Schuh et al. 2002; Pope and Liu 2004; Casida and Quistad 2005). A possible reason for the departure of these proposed mechanisms from the traditional mechanism of OP toxicity may be that low toxicity OP compounds can react with more sites of toxicity because of the high doses of these compounds required to produce toxic

effects in comparison to high toxicity OP compounds for which a single specific site of toxicity is more plausible. The purpose of this report is to examine the hypothesis that AChE inhibition is the mechanism of acute toxicity of OP compounds by mathematically modeling the in vivo lethal effects of highly toxic OP compounds and determining the amount of variation in OP toxicity that is explained by AChE inhibition.

Materials and methods

Animals

Male Sprague–Dawley rats (230–250 g), Hartley guinea pigs (330–360 g), New Zealand rabbits (2.5–3.0 g) and CD-1 (ICR) mice (22–26 g) were obtained from Charles River Laboratories (Wilmington, MA), quarantined upon arrival and screened for evidence of disease. Animals were allowed free access to food and water before and after administration of organophosphorus (OP) compounds or drugs. Animal rooms were maintained at 20–22°C and 50% relative humidity with at least ten complete air changes per hour. All animals were on a 12-h light/dark full spectrum lighting cycle with no twilight. All animal procedures described in this report were performed in accordance with the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Resources, National Research Council, in accordance with the stipulations mandated for an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility.

Chemicals

Soman, sarin, VX, cyclosarin and tabun were obtained from the Edgewood Chemical and Biological Center (Aberdeen Proving Ground, MD) and were > 98.6% pure as determined by ^{31}P-nuclear magnetic resonance spectroscopy. Paraoxon, atropine sulfate and diisopropylfluorophosphate (DFP) were purchased from Sigma Chemicals (St Louis, MO). Pralidoxime chloride (2-PAM) and obidoxime dichloride (OBID) were purchased from Ayerst Laboratories (New York, NY) and Duphar (Amsterdam, Netherlands), respectively. All drugs were USP grade. Drugs and OP compounds were administered as solutions in saline with injection volumes ≤ 1 ml/kg.

OP compound toxicity

Median lethal doses (LD_{50}) were determined from the 24-h mortality of animals receiving OP compounds by subcutaneous (sc) administration. LD_{50} values were calculated by probit analysis (Finney 1971) of mortality fractions with at least six doses of each OP compound and ten animals per dose. Significant differences between probit slopes of dose–response curves were identified by a two-sample t-test. Differences were considered significant if $P < 0.05$.

Oxime efficacy

Efficacy of oximes against OP compounds was determined from the 24-h mortality of guinea pigs receiving oxime (145 μmol/kg) and atropine (50 μmol/kg) by intramuscular (im) injection 1 min after sc administration of an OP compound. An oxime'9s efficacy was expressed as a protective ratio, which is the ratio of the OP LD_{50} in oxime/atropine-treated animals divided by the OP LD_{50} in untreated animals. Protective ratios were determined using a stage-wise method (Maxwell et al. 1997).

Mathematical modeling

Mathematical models of relationships between in vivo and in vitro variables were analyzed by linear regression and graphed with SigmaPlot 2000. The fraction of total variation explained by each mathematical model was determined from its regression correlation coefficient (r) where r^2 = (explained variation)/(total variation).

Results

To compare the in vivo dose–response curves for a variety of highly toxic OP compounds, the mortality dose–response curves for VX, soman, cyclosarin, sarin, tabun, paraoxon and DFP were determined in guinea pigs. The LD_{50} values and probit slopes characterizing the dose–response curves for these OP compounds are shown in Table 1. Although these OP compounds exhibited LD_{50} values that varied 439-fold, their probit slopes were steep (9.6–11.3) and were not significantly different.

To compare OP dose–response curves in different species, the mortality dose–response curves for one OP compound, soman, were also determined in mice, rats and rabbits (Fig. 1). For comparison of soman dose–response curves in rodents and non-human primates, Fig. 1 also contains dose–response curves for marmosets and rhesus monkeys taken from Dirnhuber et al. (1979). Although the LD_{50} values for soman varied 15-fold among these species, the probit slopes of the

Table 1 Comparison of LD_{50} values and probit slopes for OP compounds in guinea pigs

OP Compound	LD_{50} (μg/kg) sc	Probit slope ± SE
VX	9.15 (8.01–10.3)	10.7 ± 2.55
Soman	34.1 (31.0–37.7)	9.62 ± 2.32
Sarin	44.3 (39.0–50.5)	9.95 ± 2.81
Cyclosarin	57.9 (50.3–66.5)	9.81 ± 2.77
Tabun	117 (101–134)	9.53 ± 2.30
Paraoxon	941 (790–1101)	11.3 ± 3.01
DFP	4018 (3,334–4,741)	9.85 ± 2.46

Values in parentheses are 95% confidence intervals

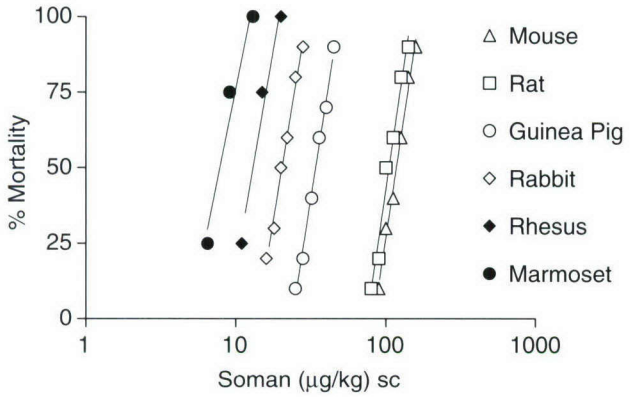

Fig. 1 Comparison of the mortality dose–response curves for soman in different species. Data for marmoset and rhesus monkey were taken from Dirnhuber et al. (1979)

soman dose–response curves were similar and as steep in these species as the probit slope for soman in guinea pigs presented in Table 1. These observations demonstrated that highly toxic OP compounds produce similar steep probit slopes regardless of the OP compound or the mammalian species in which the toxicity of the OP compounds is determined.

Evaluation of the hypothesis that AChE inhibition is the in vivo mechanism of OP toxicity was examined in Fig. 2 by regression analysis of the relationship between the bimolecular rate constants for OP inhibition of AChE (k_{AChE}) and OP LD_{50} values in rats. Regression analysis of in vitro and in vivo data taken from Maxwell (1992) showed that $LD_{50} \propto (k_{AChE})^{-½}$ with $r^2 = 0.93$, which indicated that 93% of the 3,280-fold variation in the acute toxicity of OP compounds was explained by the variation in the ability of these compounds to inhibit AChE.

Since inhibition of AChE was highly correlated with the toxicity of OP compounds, the ability of reactivation of OP-inhibited AChE to correlate with protection against the toxicity of OP compounds was also examined. The efficacy of 2-PAM (145 μmol/kg)

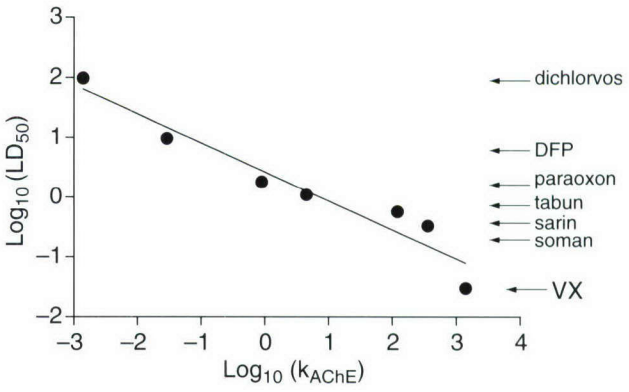

Fig. 2 Regression analysis of in vitro inhibition of AChE and in vivo toxicity of OP compounds in rats. LD_{50} values and inhibition rate constants were taken from Maxwell (1992)

and atropine (50 μmol/kg) against VX, sarin and cyclosarin was determined in guinea pigs and expressed as protective ratios (PR) in Table 2. These data were supplemented with comparable oxime efficacy data from a literature review by Dawson (1994) to increase the number of data points for a regression analysis of in vivo oxime efficacy against in vitro oxime reactivation. The supplemental efficacy data were selected on the basis that oxime PR values had to have been determined (1) in guinea pigs, (2) with a dose of atropine (46–50 μmol/kg) comparable to the dose used in the current paper and (3) with oximes for which in vitro AChE reactivation rate constants were available. These criteria resulted in the addition of the supplemental oxime PR data shown in Table 2 from Fleisher et al. (1970), Lundy et al. (1992) and Inns and Leadbeater (1983). In vitro oxime reactivation constants (k_R) were taken from Worek et al. (2002), which contained an extensive study of oxime reactivation of OP-inhibited AChE from guinea pigs.

A regression analysis of the relationship between the in vivo PR of OBID and 2-PAM at different oxime doses and their in vitro k_R values for oxime reactivation of OP-inhibited AChE is shown in Fig. 3. Efficacy of oximes against the toxicity of VX, sarin and cyclosarin in guinea pigs was expressed as PR-1, because a PR of 1 denotes an absence of oxime protection, and k_R was multiplied by the administered dose of oxime ([Oxime]) to normalize the effect of different oxime doses. Regression analysis showed that PR-1 $\propto (k_R[\text{Oxime}])^{0.56}$ with $r^2 = 0.91$, which indicated that the ability to reactivate OP-inhibited AChE explained 91% of the 23-fold variation in the efficacy of oximes against highly toxic OP compounds.

Table 2 Comparison of oxime efficacy against OP compounds in guinea pigs

OP Compound	Oxime	Oxime dose (μmol/kg)	Protective ratio (PR)	Reference for PR
VX	2-PAM	145	36.8 (27.9–46.1)	Current paper
Sarin	2-PAM	145	22.7 (17.4–29.5)	Current paper
Cyclosarin	2-PAM	145	2.6 (2.1–3.5)	Current paper
VX	2-PAM	130	25	Inns and Leadbeater (1983)
VX	OBID	40	58	Inns and Leadbeater (1983)
Sarin	2-PAM	130	38	Inns and Leadbeater (1983)
Sarin	OBID	40	59	Inns and Leadbeater (1983)
Sarin	2-PAM	43	19	Fleisher et al. (1970)
Sarin	OBID	47	42	Fleisher et al. (1970)
Cyclosarin	OBID	56	4	Lundy et al. (1992)

Values in parentheses are 95% confidence intervals

Discussion

In as much as OP inhibition of AChE in vitro is an extremely rapid and specific reaction (De Jong and Benschop 1988), it would be expected that the in vivo dose–response of an OP compound would reflect this specificity. Mortality dose–response curves for a variety of highly toxic OP compounds with sc LD_{50} values that varied 439-fold in guinea pigs exhibited similar steep probit slopes that were > 9.6. Similar monophasic steep probit slopes were also observed when the mortality dose–response curves for soman were examined in six different species whose soman LD_{50} values varied 15-fold. The consistently steep slopes of these OP dose–response curves regardless of their midpoints (i.e., LD_{50}) suggested that OP compounds are potent toxicants for which small changes in dose result in large changes in response.

Compounds with multiple mechanisms of toxicity have complex in vivo dose–response curves with shallow probit slopes that reflect the cumulative effect of multiple toxic mechanisms with different thresholds (Faustman and Omenn 2001). The slopes of the mortality dose–response curves for the highly toxic OP compounds in the current study were similar to the steep slope of the mortality dose–response curve for botulinum toxin (Rai and Van Ryzin 1981), an extremely toxic substance (i.e., LD_{50} < 0.01 μg/kg) with a specific mechanism of toxicity (Szinicz and Baskin 1999). Our analysis of the in vivo dose–response curves of highly toxic OP compounds suggested that these compounds have a single specific mechanism of acute in vivo toxicity regardless of the OP compound or the species in which OP toxicity is evaluated.

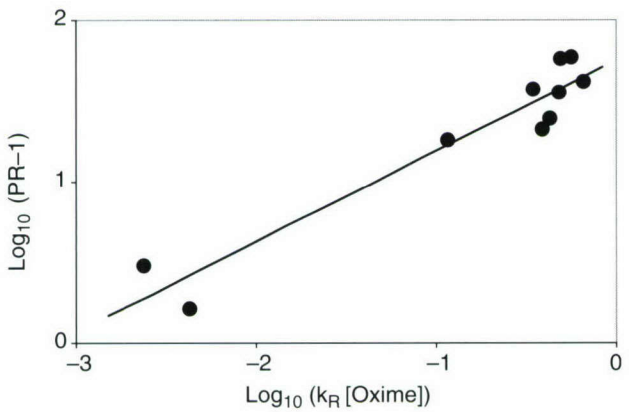

Fig. 3 Regression analysis of in vitro oxime reactivation and in vivo oxime protection in guinea pigs. PR values were taken from Table 2. Reactivation rate constants were taken from Worek et al. (2002)

Evaluation of the hypothesis that AChE inhibition is the in vivo mechanism of action of OP compounds was examined by two approaches. One approach examined the mathematical relationship between in vivo OP LD_{50} values and in vitro OP inhibition of AChE (k_{AChE}). Regression analysis of this relationship indicated that 93% of the variation in the acute toxicity of OP compounds was explained by the variation in their ability to inhibit AChE. The other approach was to examine the mathematical relationship between in vivo oxime efficacy against OP compounds and in vitro oxime reactivation of OP-inhibited AChE. Regression analysis of this relationship indicated that 91% of the variation in the protection of oximes against highly toxic OP compounds was explained by the ability of oximes to reactivate OP-inhibited AChE. The consistency between the ability of AChE inhibition to explain > 90% of the variation in toxicity of OP compounds and the ability of AChE reactivation to explain > 90% of the variation in oxime efficacy against OP toxicity presents a compelling argument that AChE inhibition is the primary mechanism of OP toxicity.

The best explanation for this variety of observations was that the primary mechanism of in vivo toxicity for highly toxic OP compounds is the inhibition of AChE, and the residual unexplained variation in OP toxicity that might be explained by other mechanisms represents < 10% of the total variation across a 3,280-fold range of OP toxicity.

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